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VARIATION IN MITOCHONDRIAL DNA OF FOUR SPECIES OF MIGRATORY RAPTORS[1]

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ABSTRACT.—Four species of North American raptors, the Sharp-shinned Hawk (*Accipiter striatus*), Cooper's Hawk (*A. cooperii*), Red-tailed Hawk (*Buteo jamaicensis*), and American Kestrel (*Falco sparverius*) are migratory and utilize established flyways for summer and fall migrations. I used restriction-fragment analysis of mitochondrial DNA from individuals from each of these four species on two western and one eastern migratory flyway to test for genetic differences indicative of separation of eastern and western populations. Although the differences were small, western migratory Red-tailed Hawks possessed different mtDNA haplotypes than eastern individuals. There were no consistent differences between eastern and western individuals of the other three species. Further analyses of widespread migratory species of raptor are clearly indicated using other appropriate techniques.

KEY WORDS: *American Kestrel*; Falco sparverius; *Cooper's Hawk*; Accipiter cooperii; *Sharp-shinned Hawk*; Accipiter striatus; *genetic variation*; *mitochondrial DNA*; *RFLP*.

VARIACIÓN DEL ADN MITOCONDRIAL EN CUATRO ESPECIES DE RAPACES MIGRATORIAS

RESUMEN.—Cuatro especies de aves rapaces norteamericanas (*Accipiter striatus, A. cooperii, Buteo jamaicensis,* y *Falco sparverius*) son migratorias y utilizan rutas establecidas para su migración de primavera y otoño. Utilizé un análisis de ADN mitocrondial de fragmento restringido de individuos de cada una de estas cuatro especies en dos rutas de migración del oeste y una del este, para probar las diferencias genéticas indicativas de una separación entre las poblaciones de este y el oeste. Aunque las diferencias fueron pequeñas, los gavilanes colirojos migratorios del oeste tuvieron Haplotipos del AND mitocondrial dierentes a los del este. No hubo diferencias consistentes entre individuos del resto de especies. Un análisis mas amplio de especies migratorias de aves rapaces son claramente indicados mediante la utilización de técnicas apropiadas.

[Traducción de César Márquez]

Many North American raptor species are migratory and may fly distances of hundreds to thousands of kilometers to their wintering habitat. Some are separated into distinct geographical races or subspecies; the Red-tailed Hawk (*Buteo jamaicensis*) for example, has had a total of 14 races described for northern and central America (Preston and Beane 1993). Other raptor species are not as geographically differentiated, but one to several geographic races may be recognized. Migratory birds have been traditionally assumed to have high levels of gene flow due to their high mobility (Arguedas and Parker 2000), presumably resulting in low levels of genetic diversity, and they have been relatively ignored in phylogeographic studies (Kimura et al. 2002). A few studies of migratory birds have found recognizable markers in some populations (Haig et al. 1997, Milot et al. 2000, Kimura et al. 2002) or a mixed pattern of population separation in shorebird species (Wenink et al. 1994, Haig et al. 1997). Species with northern distributions have also been shown to have shallow population structure owing to probable post-Pleistocene colonization events (Zink 1996).

Many studies have used the mitochondrial genome (mtDNA) or genomic DNA to assess population genetic structure (Wenink et al. 1994, Zink 1996, Haig et al. 1997, Arguedas and Parker 2000, Milot et al. 2000, Kimura et al. 2002). Restriction analyses of mtDNA involve the cleaving of the mitochondrial genome at sites varying from four to

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six base pairs in length. If the sequence of base pairs is the same, cleavage fragments of identical size are created. These differences can be visualized for comparison using agarose-gel electrophoresis (Quinn 1997). Differences in sequence between individuals at these restriction sites will result in different fragment sizes. This provides a quick assessment of a percentage of the mitochondrial genome depending on how many restriction enzymes are used.

If migratory raptors are maintaining distinct populations by returning to the same breeding grounds every spring, these differences should result in separation of mtDNA lineages. Genetic differences may not exist if migratory individuals are not maintaining distinct populations, if they have a recent history of colonization in the north, or if there has been a recent bottleneck in the populations as that found in chickadees (*Poecile* spp.; Gill et al. 1993).

The Sharp-shinned Hawk (*Accipiter striatus*), Cooper's Hawk (*A. cooperii*), Red-tailed Hawk (*Buteo jamaicensis*), and American Kestrel (*Falco sparverius*) are found throughout North America with many populations exhibiting north-south migration in response to seasonal changes. Although migratory birds travel long distances and experience mixing of individuals from different areas on the wintering grounds, in some cases, such as Dunlins (*Calidris alpina*; Wenink et al. 1993, 1996, Haig et al. 1997) and Snow Geese (*Chen caerulescens*; Quinn 1992), breeding populations remain distinct. This is not true for all species; shorebirds with differing mating systems and degrees of natal philopatry exhibited varying degrees of population differentiation (Haig et al. 1997). In raptors, which generally exhibit natal philopatry (Newton 1979), North American populations may remain distinct. Here I address the question, are migratory raptors maintaining distinct populations? If so, is this pattern consistent across different taxa? And, does it reflect variation in morphology?

In this study, I examined patterns of variation of neutral molecular markers within four species of migratory raptors to determine if they are maintaining distinct differences between eastern and western populations. Migratory individuals of all four species sampled on eastern and western flyways exhibit significant morphological differences (Pearlstine in press) and the Red-tailed Hawk is polytypic with a distinctive eastern and western subspecies (Preston and Beane 1993). I sampled individuals from two migratory routes in western North America and one on the east coast. Haplotype variation was used to provide an indication of potential population separation between raptors using different flyways.

STUDY AREA

The Goshute Mountains of Nevada and the Manzano Mountains of New Mexico are monitoring points along major raptor flyways in the west (Hoffman et al. 2002), and Cape May Point in New Jersey is situated on a major eastern flyway (Clark 1985). The Goshute and Manzano Mountain flyways are both situated along ridge systems Migrants through Cape May Point build up along the Atlantic coastline and funnel along the southern New Jersey peninsula to cross the ocean at the point. Based on available band returns, breeding grounds are thought to be north of the western flyways (Smith et al. 1990, Hoffman et al. 2002) and north and east of the eastern flyway (Clark 1985, W. Clark pers. comm.). Goshute and Manzano migrants travel each fall to wintering grounds in central and western Mexico, a distance that may be as much as twice that of eastern migrants, which tend to remain in the southeastern United States (Clark 1985, Smith et al. 1990, and W. Clark pers. comm.).

METHODS

I took blood samples from 142 individual birds during migration in the fall of 1993–95 on each of the three flyways. These were frozen or preserved in a lysis buffer (100 mM Tris—HCL, pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) and DNA was isolated using a standard phenol-chloroform extraction. The gene region of interest included the ND2 gene, five tRNA genes, and part of the COI gene (about 2150 base pairs). Samples were amplified by PCR using primers and PCR protocols from Riddle et al. (1993; also see Hillis and Moritz 1990). These samples were digested with 18 four-base restriction enzymes (Promega, Inc. Madison, WI U.S.A.; Table 1) Samples were electrophoresed through 1.2% agarose gel and fragments were visualized with ethidium bromide Resultant restriction digest fragments were sized relative to a standard 1-kb ladder-molecular-weight marker. Fragment patterns were used to infer restriction sites (Dowling et al. 1990). Fragments resulting from digestion with a single restriction enzyme were assumed to result from cleavage at identical sites if the fragments were of equal size. The composite haplotype for each individual was recorded using a different letter for distinct patterns for each restriction endonuclease. I calculated genotypic divergence following the method of Upholt (1977). Haplotype diversity was calculated following the methods outlined in Ball and Avise (1992). All work was done in the laboratory of Drs. B.R. Riddle and A.P. Martin at the University of Nevada, Las Vegas, NV.

RESULTS

The ND2/CO1 region of the mtDNA was estimated to be ca. 2200 bp or ca. 7.5% of the 16 500 bp mitochondrial genome. I scored a total of 35 sites in all individuals giving information on about

Table 1. Haplotype composition, overall frequency, and frequency within flyways. Common haplotypes are indicated by the letter A for Sharp-shinned Hawks, Red-tailed Hawks, and American Kestrels; for the Cooper's Hawk, the common haplotype is indicated by the letter B, where it is different from that of Sharp-shinned Hawks for the same restriction enzyme.

Haplotype	Haplotype Frequency			
	Overall	Goshute Mtns.	Manzano Mtns.	Cape May Point
Sharp-shinned Hawks[a]				
Haplotype 1 A A A A A A A A A A A A A A A A A A	0.82	0.73	1.00	0.75
Haplotype 2 A A A A A A A A B A A A B A A A A	0.03	—	—	0.08
Haplotype 3 A A A A A A A A A A A B A A A A A A	0.03	0.09	—	—
Haplotype 4 A A A A A A A A A A A B A A A A A	0.12	0.18	—	0.17
Cooper's Hawks[b]				
Haplotype 1 B B A B A B A B C A A B B B B B	0.86	1.0	0.87	0.71
Haplotype 2 B C A B A B A B C A A B B B B B	0.14	—	0.13	0.29
Red-tailed Hawks[c]				
Haplotype 1 A A A A A A A A A A A A A A A A A	0.27	0.63	0.25	—
Haplotype 2 A B A A A A A A A A A A A A A A A	35	0.37	0.75	—
Haplotype 3 A B A A A A A A A A A A A A A B	0.38	—	—	1.00
American Kestrels[d]				
Haplotype 1 A A A A A A A A A A A A A A A A A	0.97	1.0	1.0	0.91
Haplotype 2 A A A A A A A B A A A A A A A	0.03	—	—	0.09

[a] Enzymes used: *Aci*I, *Alu*I, *Ava*II, *Bfa*I, *Bsp*1286I, *Bst*NI, *Bst*UI, *Dde*I, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Hpa*I, *Mbo*I, *Nla*III, *Rsa*I, and *Taq*I.
[b] Enzymes used: *Aci*I, *Alu*I, *Bfa*I, *Bsp*1286I, *Bst*NI, *Bst*UI, *Dde*I, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Hpa*I, *Mbo*I, *Nla*III, *Rsa*I, and *Taq*I.
[c] Enzymes used: *Aci*I, *Alu*I, *Ava*II, *Bfa*I, *Bsp*1286I, *Bst*NI, *Bst*UI, *Dde*I, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Mbo*I, *Nla*III, and *Rsa*I.
[c] Enzymes used: *Aci*I, *Alu*I, *Ava*II, *Bfa*I, *Bsp*1286I, *Bst*NI, *Bst*UI, *Dde*I, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Mbo*I, *Nla*III, and *Rsa*I.
[d] Enzymes used: *Aci*I, *Alu*I, *Ava*II, *Bfa*I, *Bsp*1286I, *Bst*NI, *Dde*I, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Hpa*I, *Mbo*I, *Nla*III, and *Taq*I.

1.3% of the mtDNA genome, except for the American Kestrel (see below).

Sharp-shinned Hawks. I tested 34 individuals and found no apparent genetic structuring of populations. Twenty-eight individuals (82%) from all three flyways shared the same haplotype (Table 1). Of the 17 four-base restriction enzymes used, four yielded different fragment lengths in a total of six individuals. Rare haplotypes were found in one Cape May migrant and one Goshute migrant. Two individuals from Cape May Point and two from the Goshute Mountains exhibited a third unique haplotype. All haplotypes differed by only one restriction site except for one Cape May migrant that differed by two. Haplotypes differences are small and haplotype diversities are low. Mean genetic distance (p) for the Sharp-shinned Hawks was 0.0003 with a haplotype divergence of 0.32 (Table 2).

Cooper's Hawks. I examined 44 individuals and found no apparent genetic structuring of populations. Thirty-six (82%) shared the same haplotype (Table 1). Of the 16 restriction enzymes used, only one alternative haplotype that differed by a single restriction site was identified. Two Manzano migrants and four Cape May migrants exhibited this haplotype. The frequency of haplotypes was proportionately different between flyways but these differences are slight. Mean distance of haplotypes was 0.003 and divergence between haplotypes was 0.24 (Table 2).

Comparison of Sharp-shinned and Cooper's Hawks. These species differed from each other for 11 of the 15 enzymes used, ca. 17 sites (Table 1). Mean genetic distance between the two species was 0.06. Given the divergence detected between species, the low within species diversity is not due to lack of ability to detect variation for the restriction enzymes used. Relative to the scale of divergence between species, the divergence within species appears to be very recent.

Red-tailed Hawks. For 26 individuals tested there was evidence of genetic population structure between individuals using eastern and western flyways. From the 15 four-base restriction enzymes

Table 2. Summary of genetic characteristics and haplotype diversity. Genotypic diversity = $(n/[n-1])\ (1-3f_i^2)$, where f_i is the frequency of the ith mtDNA haplotype (Ball and Avise 1992). $p = 1 - [0.5(-F + \{F^2 + 8F\}^{0.5})]^{1/r}$, where $F = 2N_{xy}/(N_x + N_y)$ and r is the number of base pairs in the enzyme's recognition site (Avise 1994). All enzymes used recognized four base pairs.

Species	N	No. Haplotypes/ Individual	Genotypic Diversity	Nucleotide Diversity (p)
Sharp-shinned Hawk	34	0.12	0.32	0.0004
Cooper's Hawk	44	0.05	0.24	0.0003
Red-tailed Hawk	26	0.12	0.69	0.0090
American Kestrel	38	0.05	0.06	0.0001

used, two (*Alu*I and *Rsa*I) yielded different fragment lengths. Of the haplotypes identified, one was specific for the Cape May migrants and differed from one of the western haplotypes by one restriction site (Table 1). In the western migrants, there were two mtDNA haplotypes, both of which showed variation in frequency between Goshute and Manzano migrants. Haplotype A was found in 27% of the individuals, and haplotype B in 35% of the individuals. The third haplotype constituted 38% of the samples and was found only in individuals from Cape May Point. This geographic structure, although slight, indicates a consistent separation of eastern and western migratory Red-tailed Hawks. Individuals from the two western flyways did not differ in haplotype composition, but did in frequency of haplotypes. Mean genetic distance was 0.09 and haplotype diversity was 0.686 (Table 2).

American Kestrels. I tested 38 individuals and found no apparent population genetic structure. Fifteen restriction enzymes recognized ca. 30 restriction sites. This represents a little less than 1.2% of the mtDNA genome. Birds from all flyways had a single, common haplotype (Table 1) with the exception of one individual from Cape May Point that differed from the rest by one restriction site. Genetic distance is 0.0001 and haplotype diversity is 0.06 (Table 2).

Discussion

Patterns of mtDNA restriction-fragment length polymorphisms that may be indicative of phylogeographic structure were found only in the Red-tailed Hawk. In this species, the eastern individuals all differed by a single restriction site from all western individuals although haplotype diversity was low. This corresponds with distributions of subspecies *calurus* and *borealis* (Preston and Beane 1993); all individuals exhibited morphological characters consistent with subspecies designations for their geographic area. Other studies have found eastern/western population differences in migratory warblers (Milot et al. 2000, Kimura et al. 2002) and European Rock Partridges (Lucchini and Randi 1998).

Although I found substantial differences between the two species of *Accipiter*, no population structure was detected within Sharp-shinned and Cooper's hawks. I found similar results for American Kestrels. There may be no actual population structure in these species or the methods used may not have been sensitive enough to detect the presence of different mtDNA haplotypes. These three species are all characterized by a number of subspecies, but only one occurs within the area covered by this study.

Genetic distances within and between species were within the range of distances found for other North American bird species with a primarily northern distribution, such as chickadees (Gill et al. 1993) and redpolls (*Carduelis* spp.; Seutin et al. 1995), and for bird species with a wide North American distribution (Ball and Avise 1992, Zink 1996). Differences were low compared with two species of migratory warblers, Yellow Warbler (*Dendroica petechia*; Milot et al. 2000), and Wilson's Warbler (*Wilsonia pusilla*; Kimura et al. 2002). As in this study, some bird species with a northern distribution do not exhibit clear phylogeographic structure (e.g., Avise et al. 1992, Gill et al. 1993, Seutin et al. 1995), whereas others do (Van Wagner and Baker 1990, Zink 1994, Lucchini and Randi 1998).

Future work on the population structure of raptors is clearly indicated. The application of newer

techniques such as microsatellites, which are appropriate for this level of study (McDonald and Potts 1997, Arguedas and Parker 2000, Baker 2000, Milot et al. 2000), should be applied. Also, further investigation of the control region may serve to confirm the results of this restriction fragment length polymorphism (RFLP) study (Wenink et al. 1994, Baker and Marshall 1997) and perhaps provide more information on geographic population structure of North American raptors. Lucchini and Randi (1998) used mtDNA control region sequencing for population level studies of Rock Partridges in Europe and found that populations have remained separate since glacial recolonization. A study of shorebirds using randomly amplified polymorphic DNA (RAPD) analysis assisted in assigning individuals of some species to breeding locations (Haig et al. 1997). Further investigation into the genetic basis of subspecies designations in Red-tailed Hawks may also provide important information on the possible genetic basis of morphological differences.

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